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Drying Made Easy

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Chapter 1

General Introduction

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1. Introduction

Most vaccines and therapeutic proteins are inherently unstable in liquid form because they are prone to chemical and physical degradation, such as oxidation, deamidation and aggregation in response to temperature and pH fluctuations [1, 2]. Therefore, these biopharmaceuticals have to be handled under refrigerated conditions (2-8 °C), with far-reaching consequences. These consequences include high costs to maintain the cold chain and high vaccine losses if it is disrupted or due to inappropriate storage and possible use of vaccines with suboptimal quality. Maintenance of cold chain is challenging, especially in developing countries. This contributes to complexing of logistics for transport and distribution [3], further adding financial burden to immunization and other health programs. A potential successful strategy to stabilize biopharmaceuticals, like vaccines and therapeutic proteins, is to dry them in presence of stabilizing excipients. Removal of water can improve the stability of these biopharmaceuticals due to decreased mobility and prevention of degradation pathways that are facilitated by water [4, 5]. Thus, dried vaccines or therapeutic proteins that are stable at ambient temperature and that could reduce the dependency on the cold-chain are highly desirable. Additionally, dried vaccines may attain an extended shelf life, which holds great potential for stockpiling in case of pandemics or bioterrorism threats [6].

There are several drying techniques available for drying biologicals that includes freeze drying, spray drying, spray freeze drying, foam drying and supercritical drying (**Table 1**). These techniques introduce varying stress, which may affect the product recovery and stability. Moreover, the dried material produced using these techniques produces material with significantly different characteristics. The most commonly used drying method for vaccines and therapeutic proteins is freeze-drying [7]. The formulation is frozen and the water is removed by sublimation at low pressure. This is followed by desorption of water bound to the active pharmaceutical ingredient, resulting in a dried cake in the final container. Before administration the cake is reconstituted, usually with water [8].

An alternative to freeze-drying is spray drying. Unlike freeze-drying, there is no freezing step, thus the damage related to this step, if any, is avoided. The edge of spray drying resides in its ability for powder particles to be engineered to desired requirements, which can be used for various routes of administration including pulmonary, intranasal, intradermal and sublingual routes [9]. Although several spray dried vaccines and therapeutic proteins have shown encouraging preclinical results, the number of these formulations that have been tested in clinical trials is limited, indicating a relatively new area of vaccine and therapeutic protein formulation and delivery.

Table 1: Overview of drying processes used to produce vaccines and therapeutic proteins.

Drying Technique	Challenges encountered from equipment design, processing and product quality perspective	Advantages	Ref
Freeze drying	i) Heterogeneity in drying ii) Use of conservative cycles resulting in long processing times iii) Lack of evolution in equipment design, leading to limited heat and mass transfer iv) Cake requires reconstitution v) Large space and maintenance requirements	i) Established technology ii) Equipment widely available iii) Straightforward asceptic processing	[10-12]
Foam drying	i) Rapid boiling/boil over, freezing during foaming ii) Inability to control higher temperatures, pressures during foam formation iii) Long duration of drying to reduce water content iv) Bulky product compared to product obtained from other drying techniques	i) Drying (under vacuum) at near ambient conditions ii) Does not require freezing iii) Feasible for temperature sensitive products	[13-15]
Spray drying	i) Significant shear stress during atomization ii) Exposure of dried material to high temperatures in the collection vessel iii) Higher water contents in comparison to freeze drying iv) Asceptic processing difficult	i) Uniform product format ii) Particle engineering / encapsulation feasible iii) Versatile product filling options	[12, 16, 17]
Spray freeze drying	i) Significant shear stress during atomization and freezing ii) Difficult to scale up iii) Asceptic processing difficult	i) Uniform particles ii) Feasible with temperature sensitive products	[18, 19]
Supercritical fluid drying	i) Impact of shear, CO_2 pressure and organic co solvents (during processing and residual levels post processing) on protein stability ii) High equipment costs iii) Asceptic processing difficult	i) Drying at ambient temperatures ii) Wide particle formats feasible	[20, 21]

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2. Aim

The main goal of the thesis is to investigate the applicability of the spray drying technique to various biopharmaceuticals including vaccines and therapeutic proteins.

More specifically, the objectives were:

- To understand the impact of the spray drying process on product characteristics and quality by applying a structured Design of Experiment approach using a model viral vaccine (Influenza vaccine).
- To evaluate the potential of different excipients, in minimizing the loss in potency for a thermolabile viral vaccine (Sabin Inactivated Polio vaccine), during spray drying and subsequent storage.
- To develop a spray-dried, stable, powder of outer membrane vesicles of pertussis vaccine and evaluate the efficacy after pulmonary immunization in mice.
- Finally, to extend the understanding of spray drying process to stabilize therapeutic proteins like monoclonal antibodies and compare it with the traditional freeze-drying.

3. Outline of the thesis

In **Chapter 2,** the current status and novel developments of spray drying as a method for drying vaccines is reviewed. We focused on the impact of process stress on vaccine integrity and the application of excipients in spray drying of vaccines. This chapter further addresses the spray drying process and formulation optimization strategies based on Design of Experiment approach. Finally, the potential and limitations of delivery routes for powder vaccines are discussed.

In **Chapter 3,** the Design of Experiment approach was investigated to systematically screen and optimize the spray drying process parameters to stabilize whole inactivated influenza virus (WIV) vaccine. The process parameters inlet air temperature, nozzle gas flow rate and feed flow rate and their effect on WIV vaccine powder characteristics such as particle size, residual moisture content, powder yield and antigenicity were investigated. The vaccine powders were stored at elevated temperatures (60°C for 3 months) and were compared to the liquid WIV vaccine formulation in terms of antigenicity.

With insights into the spray drying process control for stabilizing an inactivated influenza vaccine, we applied this knowledge in **Chapter 4** to develop a spray dried Sabin inactivated polio vaccine formulation. This was done by strategic excipient screening to minimize the loss of intact sIPV, so-called D-antigen, upon drying (and subsequent reconstitution), and improving the thermostability of sIPV. Furthermore, a fractional factorial design was applied around the most promising formulations to elucidate the contribution of each excipient in stabilizing D-antigen during drying.

The above accomplished research contributed to the spray drying technology for viral vaccine stabilization. With good understanding of process and formulation excipients, we focused our further research on bacterial vaccines. Thus, in **Chapter 5,** we designed a dry powder formulation of outer membrane vesicles of Pertussis vaccine (omvPV) by spray drying with an objective to potentially induce a mucosal immune response on pulmonary immunization. We formulated a dry omvPV powder preserving the structural integrity and biological activity of the vaccine in comparison to the liquid omvPV formulation. In addition, a stability study was performed by storage of powder omvPV at elevated temperatures (4 weeks at 65°C). Mice were immunized with reconstituted powder omvPV and compared the induction of protective immunity markers next to the protection efficacy after intranasal *B. pertussis* challenge to pulmonary and subcutaneous immunization of liquid omvPV.

Finally, we investigated the spray drying technology to produce formulations of therapeutic proteins, a monoclonal antibody Infliximab. Thus in **Chapter 6**, we investigated the stabilization of Infliximab by spray drying and compared it with freeze-drying (in vials as well as in bulk in Lyoguard trays). Freeze-drying in Lyoguard trays and spray drying are of interest because of the potential to scale up the drying process for bulk powder production, that could

potentially facilitate the development of an oral dosage form as alternative for the current intravenous administration route. This study focusses on excipients and excipient combinations for production of thermostable Infliximab powder formulations.

In **Chapter 7**, the results of the research described in this thesis are summarized and the perspectives are discussed.

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